

Synthesis of tripeptides as potent *Yersinia* protein tyrosine phosphatase inhibitors

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Received 9 May 2005; revised 6 June 2005; accepted 6 June 2005

Available online 20 July 2005

Abstract—We report the synthesis of a series of monoanionic phosphotyrosyl (pTyr) mimetic-containing tripeptides based on ‘Fmoc-Glu(OBn)-Xxx-Leu-amide’ (where Xxx = pTyr mimetic) and their N-terminally modified derivatives. The inhibitory potencies of compounds were tested against YopH and human PTP1B enzymes. Several compounds exhibited noteworthy activity against both YopH and PTP1B. Among the N-terminally modified analogues, 5-methylindole derivative **30** was found to be the best moiety to replace base-labile Fmoc group. A mode of binding with YopH is proposed for tripeptides **21**, **30**, and **31**.
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Yersinia pestis, a Gram-negative bacillus, is the causative agent of plague.^{1,2} The pathogenicity of *Yersinia* relies on the activity of a bacterial virulence factor called YopH, a eukaryotic-like protein tyrosine phosphatase (PTP). YopH disrupts host signal transduction processes by dephosphorylating a variety of proteins associated with the focal adhesion. This interferes with the immune response of the host, including phagocytosis.³ Because of its potential use for bioterrorism, YopH has recently emerged as an important target for antiplague therapeutics.

Protein tyrosine phosphatases (PTPs) constitute a large family of signaling enzymes. Deregulation of PTP activity can play a role in a number of diseases including diabetes, cancer, and dysfunction of the immune system. For example, PTP1B dephosphorylates the insulin receptor and causes resistance to insulin. Thus, it has been implicated in the development of type II diabetes.⁴ In recent studies, a PTP1B knockout mouse exhibited an increased insulin

sensitivity and resistance to diet-induced obesity. As a result, PTP1B is now commonly accepted as a potential target for the treatment of type II diabetes and obesity.^{5,6} Since PTPs are important in a wide variety of biological processes, there is currently significant interest in these enzymes as targets for therapeutic intervention and a great deal of effort is being invested toward the development of potent and specific PTP inhibitors.⁷

Progress has recently been made on the development of highly potent and specific PTP inhibitors, offering promise in finding effective candidates that may serve as starting points for drug development aimed at a variety of diseases.^{8–13} Our ongoing efforts toward development of novel PTP inhibitors have been based on the fact that pTyr residues play major roles in PTP substrate binding. Using nonhydrolyzable pTyr mimetics displayed in an EGFR-derived peptide platform ‘Ac-Asp-Ala-Asp-Glu-Xxx-Leu-NH₂’ (where Xxx = pTyr mimetic), we previously examined the inhibitory potencies against PTP1B.^{14–16} In particular, recent studies using the truncated tripeptide platform ‘Fmoc-Glu-Xxx-Leu-amide’ have shown that employment of a monoionic 4-(carboxymethoxy)Phe residue as pTyr mimetic combined

Keywords: Tripeptides; Bioterrorism; pTyr mimetics; YopH; PTP1B.

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with Glu-OBn ester in the C-1 position endows the resulting derivatives with considerably enhanced inhibitory potency against PTP1B and YopH.^{17–23} This was encouraging, since enhanced cell membrane permeability would be expected to result from decreased charge. To date, however, investigations of this tripeptide series carrying other monoanionic pTyr mimetics have not been reported. Additionally, it was of interest to examine the effects of further modifications to the N-terminus of the tripeptides. Therefore, the current study was undertaken to examine a new tripeptide series based on a similar platform but carrying monocarboxy pTyr mimetics and/or N-terminal structural modifications. Finally, docking studies were carried out on compounds **21**, **30**, and **31** to investigate molecular interactions that may lead to enhanced YopH inhibition.

Synthesis of tripeptides was accomplished using Rink amide resin under standard Fmoc-based solid-phase protocols as reported previously.^{22,23} Protected pTyr mimicking residues **1–4** (Fig. 1) were prepared according to the reported procedures^{24,25} and incorporated to the tripeptide sequence 'FmocHN-Glu(OBn)-Xxx-Leu-amide'. The N-terminally modified analogs **25–34** were prepared by piperidine-mediated N-Fmoc removal followed by capping using acids **5–7**, **9–14**, or sulfonyl chloride **8** (Fig. 2).

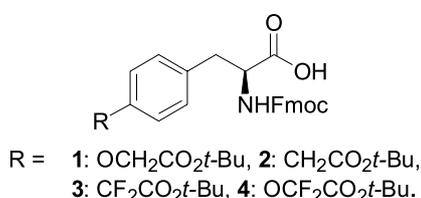


Figure 1. Structures of pTyr mimetic reagents.

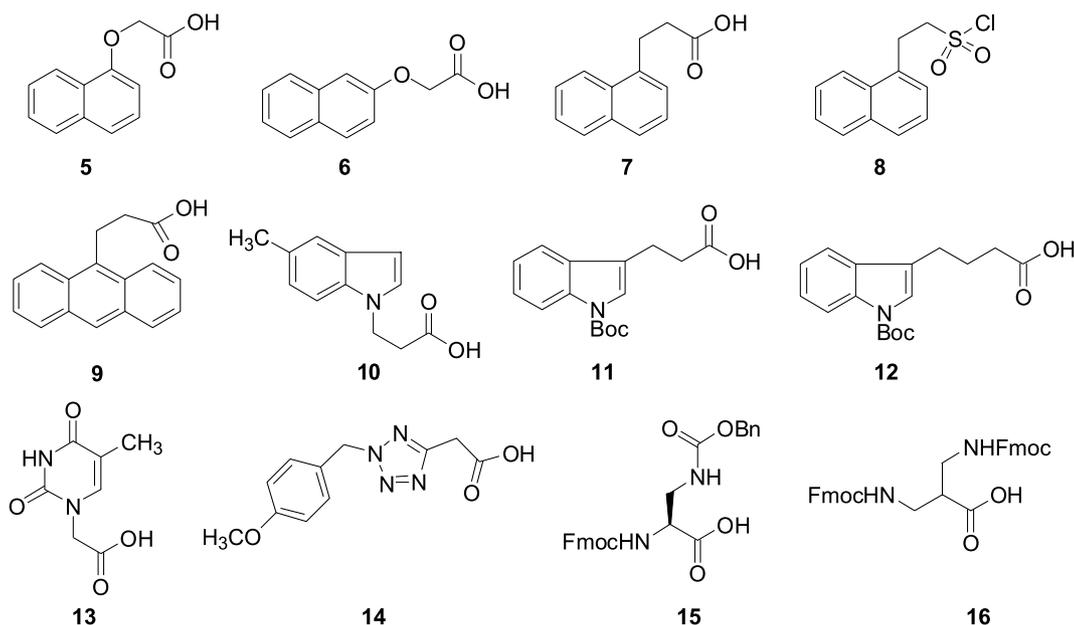
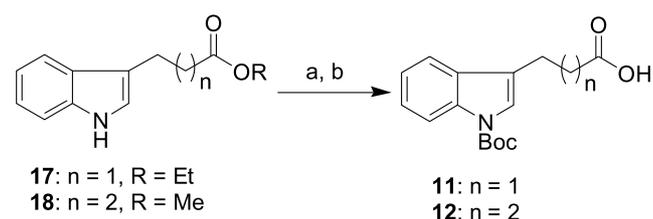
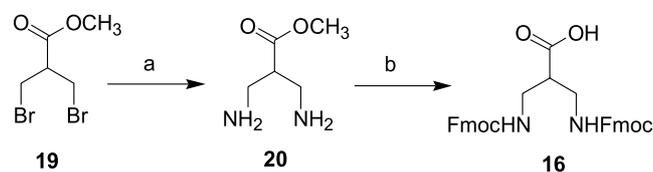


Figure 2. Reagents used for N-terminal modification of **21** following N-Fmoc removal.

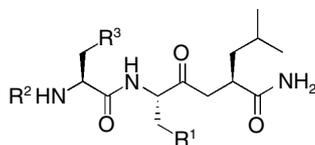
Most capping reagents were commercially available except for **10–12** and **14**. 3-(5-Methyl-indol-1-yl)-propionic acid **10** and [2-(4-methoxy-benzyl)-2H-tetrazol-5-yl]-acetic acid **14** were readily prepared following the reported procedures.^{26,27} As shown in Scheme 1, indolyl acids **11** and **12** were prepared from the corresponding indolyl esters **17** and **18** by Boc protection followed by saponification. Without amine protection, the desired indolyl analogs could not be obtained. For analog **35**, commercially available Fmoc-Dap(OBn)-OH **15** (where 'Dap' indicates diamino propionic acid) was employed, instead of Fmoc-Glu(OBn)-OH, in the C-1 position of 'FmocHN-Glu(OBn)-Xxx-Leu-amide.' Similarly, symmetric amino acid-containing **36** was prepared by use of **16**, which was synthesized from bis-bromo ester **19** (Scheme 2). Azidation followed



Scheme 1. Reagents: (a) (Boc)₂O, TEA, DMAP, AcCN; (b) LiOH, THF:H₂O = 1:1.



Scheme 2. Reagents: (a) i—NaN₃, DMF; ii—H₂, 10% Pd/C, 35 psi, HCl/MeOH; (b) i—Fmoc-OSu, NaHCO₃, aqueous dioxane; ii—concd HCl, aqueous dioxane, 50 °C.

Table 1. Inhibitory potencies of tripeptides (**21–36**) against YopH and PTP1B^a

Compound	R ¹	R ²	R ³	IC ₅₀ (μM)	
				YopH	PTP1B
21			-CH ₂ CO ₂ Bn	1.8 ± 1.0 ^b	2.9 ± 1.3 ^b
22			-CH ₂ CO ₂ Bn	2.4 ± 1.4	4.6 ± 1.8
23			-CH ₂ CO ₂ Bn	2.5 ± 1.6	3.1 ± 1.6
24			-CH ₂ CO ₂ Bn	1.9 ± 1.3	3.8 ± 1.8
25			-CH ₂ CO ₂ Bn	20 ± 4	13 ± 4
26			-CH ₂ CO ₂ Bn	30 ± 6	23 ± 5
27			-CH ₂ CO ₂ Bn	18 ± 2	12 ± 3
28			-CH ₂ CO ₂ Bn	22 ± 6	24 ± 7
29			-CH ₂ CO ₂ Bn	12 ± 2	6.4 ± 0.8
30			-CH ₂ CO ₂ Bn	5.6 ± 1.2	4.3 ± 0.7
31			-CH ₂ CO ₂ Bn	>100	>100
32			-CH ₂ CO ₂ Bn	>100	>100
33			-CH ₂ CO ₂ Bn	>100	>100
34			-CH ₂ CO ₂ Bn	>100	>100
35			-NHCO ₂ Bn	2.1 ± 0.6	4.3 ± 0.7
36				4.0 ± 1.0	2.8 ± 0.4

^a YopH and PTP1B assays were conducted as previously reported in Refs. 22 and 23.^b Updated data in the current study.

by catalytic reduction of **19** provided the crude methyl 3-amino-2-(aminomethyl)propionate **20**,²⁸ which was then converted to bis-Fmoc-protected amino acid **16**. In the case of the anthracenyl derivative **29** and the three indolyl derivatives **30–32**, TFA:EDT:H₂O was used for final deprotection and cleavage from the resin, since use of TFA:TES:H₂O gave undesired reduction in the aromatic ring. For reverse-phase HPLC purification of the final peptides, basic or acidic conditions were employed depending on their solubilities.²⁹

The inhibitory potencies of the newly synthesized compounds were measured against YopH and PTP1B that is a prototypical mammalian PTP (Table 1). PTP1B and the *Yersinia* PTP (YopH) were expressed in *Escherichia coli* BL21 (DE3) cells and purified according to the previously published procedures.^{30,31} Enzyme inhibition assays were performed under standard assay conditions following our earlier described assay protocol.²² As reported earlier,^{22,23} the monoanionic 4-(carboxymethoxy)Phe-containing tripeptide **21** is the most potent YopH inhibitor among the 'Fmoc-Glu(OBn)-Xxx-Leu-amide' (where Xxx = pTyr mimetic) series. Accordingly, tripeptides with similar monocarboxy-based pTyr mimetics **22–24** were examined. Peptides with acetic acid- or difluoroacetic acid-based pTyr mimetics (**22** and **23**, respectively) were found to exhibit significant inhibitory effect against both YopH and PTP1B. The corresponding ether-linked derivative **24** was found to be a slightly better inhibitor, especially toward YopH. However, substitutions in the 4-position of Phe affect inhibitory activity only weakly.

In an effort to understand interactions of the tripeptides with YopH at the molecular level, molecular modeling studies were conducted based on the crystal structure of 'Ac-Asp-Ala-Asp-Glu-F₂Pmp-Leu-NH₂' bound to YopH.^{32,33} Compound **21** was docked into the active

site and minimized with the pTyr mimetic residue pointing toward the YopH catalytic P-loop. As shown in Figure 3, the pTyr mimetic residue of **21** is coordinated by ionic interactions with the side chain of the signature Arg409 residue and the main chain amides of the P-loop, which donate hydrogen bond interactions. This is similar to the pTyr residue of a peptide substrate. For backbone amides of the pTyr mimetic and the C-1 Glu(OBn) residues, additional hydrogen bonding is possible with the side chain of Asp231. Of note, the N-terminal Fmoc group of **21** fills an empty charged pocket, in which the fluorenyl ring makes π -cation interactions with the side chains of Lys225 and Arg228 and π - π stacking interaction with the side chain of Phe229.³⁴ These extensive contacts, including additional interactions with the charged pocket, may explain the tolerance observed for the substitution pattern of the pTyr mimetic residue displayed in 'Fmoc-Glu(OBn)-Xxx-Leu-amide' (where Xxx = pTyr mimetic).

A second group of peptides (**25–34**) that had replacements of the N-terminal Fmoc group with a variety of heterocycles, while maintaining the 4-(carboxymethoxy)Phe in place of the pTyr was also investigated. All naphthyl derivatives with various linkers **25**, **26**, **27**, and **28** exhibited moderate YopH and PTP1B inhibitory potencies. Acridine derivative **29**, which is similar in size to the fluorenyl-containing **21**, provided better inhibition than the corresponding bicyclic derivative **27**. The lower potency of **29** as compared to **21** may be a result of the flatness of the acridine ring, which might bind less favorably within the charged pocket. Peptide **30**, having an N-terminal 5-methylindolyl group, showed potent inhibition against both PTP1B and YopH, whereas indoles linked at the 3-position (**31** and **32**) lost activity. Molecular modeling studies suggest that the 5-methylindole moiety makes additional hydrophobic interactions with Leu263 as well as π -cation interactions with Lys225 and Arg228 (Fig. 4). However, the size of the

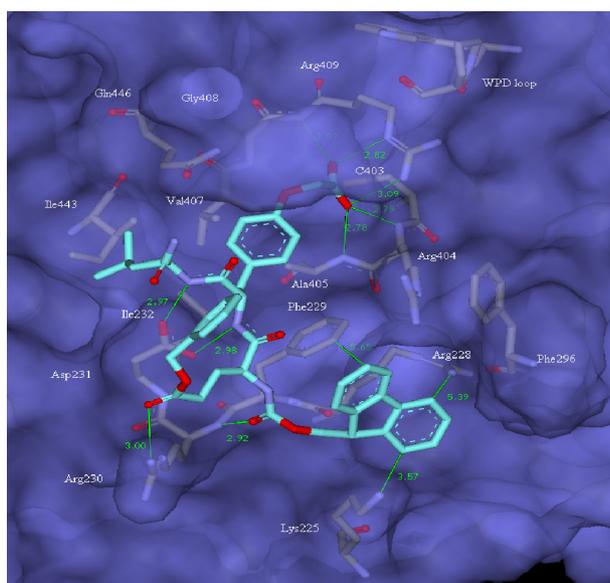


Figure 3. Computational model of **21** bound in the active site of YopH. The local solvent-accessible surface is rendered in light blue. Possible hydrogen bonds and non-bonded interactions are represented by green lines.

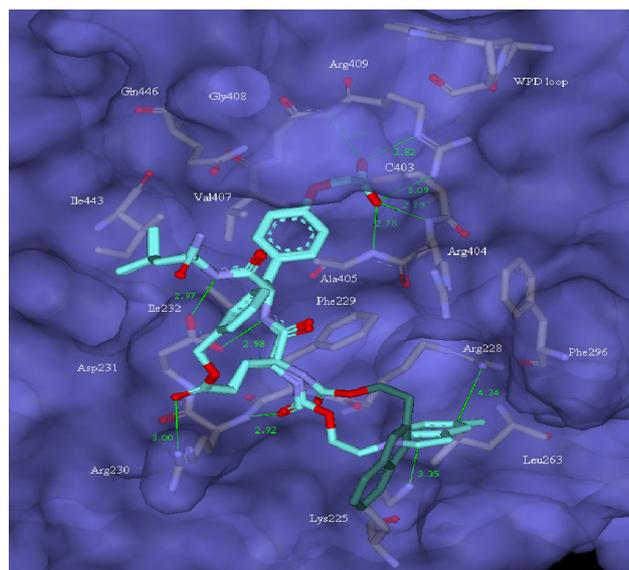


Figure 4. Overlay of the docked orientations for compounds **30** (dark green) and **31** (cyan) bound to the active site of YopH.

5-methylindole moiety of **30** is smaller than that of the tricyclic Fmoc group. Consequently, this may result in the loss of favorable π – π interactions with Phe229. Even though **30** shows lower potency than **21** (1.8 vs 5.6 μ M, respectively), the 5-methylindolyl group would be the best N-terminal moiety for replacing the base-labile Fmoc group among the current series. The superimposed conformations of **30** and **31** reveal that the orientation of the indole moiety of **31** is different from that of **30** or **21**. Binding of **31** to the YopH catalytic site might be hampered by its unfavorable steric interactions leading to loss of activity. Peptides **33** and **34** with thymine and tetrazole moieties, respectively, demonstrated poor inhibition of both YopH and PTP1B. Bis-amino tripeptides **35** and **36** were designed to explore the structural transition from asymmetric monoamino acid analog **21** to symmetric bis-amino acid analog **36** via diamino-propionic acid (Dap) containing **35**. These were well tolerated and weakly influenced the inhibition against both YopH and PTP1B.

In conclusion, modification of a promising series of previously described monoanionic pTyr mimetic-based PTP inhibitors within a similar tripeptide platform **22**–**24** resulted in significant inhibitory potencies but poor selectivity against both YopH and PTP1B similar to the previously reported **21**. Introduction of the 5-methylindolyl group at the N-terminus resulted in the potent inhibitor **30**, which indicated that the 5-methylindolyl group is an effective replacement for the base-labile Fmoc group. Molecular modeling studies examined plausible interactions of the tripeptides with YopH at the molecular level. Taken together, the current study advances the understanding of structural features that influence the PTP-inhibitory activity for this class of compounds. It also offers new possibilities for improvements in the selectivity index of tripeptides as potential leads for PTP inhibitor design.

Acknowledgments

L.W. and Z.-Y.Z. were supported by National Institute of Health Grant 1U54 AI57158. We thank Dr. Shinya Oishi for help in HPLC purifications.

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34. The crystal structure of YopH having a pTyr mimetic-containing hexapeptide bound was used as the computational model (1QZ0.pdb). The synthetic tripeptide inhibitor **21** was built from the coordinates of this pTyr mimetic-containing hexapeptide as found in the crystal structure using the Builder module in Insight II. The computational complex model was solvated using a solvent sphere of water extending 25.0 Å around the side chain sulfur atom of Cys403. The system was initially minimized using 500 steps of steepest decent and 2000 steps of conjugated gradient with a 14.0 Å nonbonded cutoff distance.
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